IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Inventor:	Crothers *et al.*	Conf. No.:	6054

Title: *Pharmaceutical Composition Comprising Fungal Cell or Fragment Thereof*

Nat'l Phase of PCT/GB2004/001072

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DECLARATION OF [Randall J. Mrsny] UNDER § 1.132

1. In 1977, I obtained a B.S. in Biochemistry and Biophysics at the University of California at Davis, I obtained a Ph.D. in Anatomy and Cell Biology at the U.C. Davis School of Medicine in 1981, and spent five years as an NIH Postdoctoral Fellow and junior staff member in Membrane Biophysics in the Institute of Molecular Biology at the University of Oregon. In 1987, I joined the drug delivery company ALZA to head the Peptide Biology Group where individuals in my laboratory examined the potential for using the company's technologies for therapeutic protein and peptide delivery. In 1990, I was recruited to Genentech, Inc. to lead a drug delivery/biology group which performed studies to better match the application of therapeutic proteins to local or targeted delivery strategies. During this time, my research focused on determining mechanisms regulating epithelial tight junction (hereafter, "TJ") structure and function for potential applications for trans-mucosal delivery of therapeutic macromolecules. In 2000, I left Genentech to start a new drug delivery company that focused on methods to move macromolecules across mucosal surfaces and into the body: Trinity BioSystems. Simultaneously, I accepted a part-time appointment at Cardiff University School of Pharmacy to continue my research in TJ biology in an academic setting. I recently left Trinity Biosystems to initiate another start-up: Unity Pharmaceuticals, a company that is exploring novel methods to treat TJ defects associated with inflammatory conditions and epithelial-derived cancers. I have also moved my part-time academic appointment to the University of Bath in the Department of Pharmacy and Pharmacology.

2. Over these years, my research studies have utilized a spectrum of approaches and focused in a variety of disciplines that has resulted in more than 100 original research and review publications. Together, this broad background has provided me with expertise in the diverse field known as drug delivery; a field that involves disciplines from materials sciences to systemic physiology. With that perspective, I have obtained a sufficient

reputation of expertise in the area of drug delivery to be voted in as president of the foremost professional society in this area: the Controlled Release Society. I was also voted by my peers to co-organize a Gordon Conference on Drug Delivery. I have served as an editor on several drug delivery-related journals, edited several books and review compilations in this area, and been invited to give numerous talks on this discipline.

3. With regard to the current area of activity, my research has extensively examined methods to dynamically regulate TJ structure and function. I have written and performed research in this area and have also reviewed numerous grants and submitted manuscripts on the topic of molecules that are capable of modulating TJ structure and function.

4. I have reviewed and fully understood both the application noted above and the prior art references cited by the Examiner in rejecting the claims of the application.

5. By way of background information, it should be noted that a number of promising drug candidates have been excluded or removed from clinical development due to their lack of efficient systemic absorption following oral administration. Those drugs that successfully pass though this development barrier are typically capable of being readily absorbed through some transcytosis pathway due to their inherent amphipathic nature. Drugs that are either not sufficiently soluble or too soluble in water tend not to be readily absorbed across the intestinal mucosa. Tight junction (TJ) structures, positioned at the apical neck of polarized epithelia, limit the uptake of solutes (i.e. applied drugs) between adjacent epithelial cells. One strategy to increase the uptake of drug candidates that are highly water soluble is to increase their potential to move between adjacent epithelial cells of the epithelial barriers that preclude their movement by transiently disrupting these TJ structures. A wide variety of methods have been identified to open TJ structures. To date, however, none of these approaches are considered acceptable for clinical use. The down-fall of these agents that manipulate TJ structures is frequently associated with overt damage to the mucosa due to their un-regulated actions at the site of application.

6. Epithelial mucosal barriers are constantly bombarded by a variety of organisms as well as materials derived from these organisms. Thus, it seems logical that dynamic, regulated interactions between mucosal barriers and these organisms (and materials derived from them) would have been established over millions of years of co-evolution. The actions and cellular re-actions to a fungal cell and/or its derivatives provide a very promising avenue to transiently increase TJ structure permeability to enhance the uptake of poorly absorbed drugs. In particular, as this dynamic relationship between a mucosal surface and yeast can result in non-toxic interactions, the actions and cellular-reactions produced by a fungal cell and/or its derivatives may overcome the overt damage problem for other approaches to open TJ structures. The application by Micap appears to have identified this dynamic relationship. To my knowledge, this has not been shown previously and would not have been anticipated as many organisms are present at epithelial surfaces and there is no way, a priori, to anticipate that one or another of these organisms might have this capability.

7. The outcome that fungal cells and/or their derivatives could open TJ structures could not have been anticipated. Evidence exists that organisms as well as materials derived from organisms that are presented to epithelial surfaces can either increase or decrease the permeability of a mucosal barrier. For example, bacterial peptidoglycans appear to function as a risk factor for Crohn's disease, a condition associated with increased permeability of the intestinal mucosa (Hugot, J-P et al 2001 Nature 411:599; Ogura Y et al 2001 Nature 411:603). Oppositely, bacterial polysaccharide A can prevent intestinal inflammation though a mechanism that up-regulates the production of cytokines known to decrease intestinal permeability (Mazmanian SK et al 2008 Nature 453:620). These examples show how organisms affect TJ structures through direct and indirect actions.

8. It is also important to point out that many materials derived from various organisms delivered to the gut as foodstuffs have no detectable affect on intestinal permeability. Thus, the outcome obtained and described in these applications must be derived experimentally and, thus, could not be expected. In fact it would have been highly likely that no effect would have been observed as fungal cells and their derivatives would be a frequent element in foodstuffs.

9. As stated above, some agents function to open the TJ (increase mucosal permeability) while others act to close the TJ (decrease mucosal permeability). Elements that might be found in foodstuffs might be present in the gut can fall into either category; in the absence of data, one skilled in the art would not be able to anticipate one outcome over the other.

10. The mucosal barrier is established by an epithelium that makes up the outermost layers of cells that face the external world. Beneath the epithelium are a number of other cells and non-cellular materials that modulate the functional nature of these epithelial cells. Some materials applied to the apical surface of epithelial cells can affect TJ function by acting directly of these cells; other materials alter the function of other cells that then secrete agents that act on epithelial TJ function (an indirect effect). Due to the complexity of the epithelium and how its function is regulated by both direct and indirect actions of applied agents, it is essentially impossible to anticipate the outcome since this outcome is a net effect of potentially competing actions. A case in point is the promising results where patients suffering from inflammatory bowel disease are treated with live eggs from the porcine whipworm (Elliot DE 2007 Int J Parasitol 37:457). The treatment with an obvious pathogenic agent to reduce inflammation is a result of the non-obvious indirect outcome of stimulating suppressing cytokines that function to reduce TJ permeability.

11. The Belanger reference describes an endoprotease from derived from *Acremonium typhinum*. Two critical issues would be required for this reference to anticipate the claims of the current invention application. The first issue is that this endoprotease actually caused the TJ to be opened in intact mucosal barriers. The second issue is that this enzyme was somehow involved in the outcomes described in the current application. With regard to the first issue – I could not find any statement in the reference that

suggested this protease could affect TJ structure/function nor could I find any statement suggesting this protease altered epithelial barrier properties. With regard to the second issue – it is my understanding that in some of the studies performed by Micap, fungal cells treated with very harsh chemical and temperatures to alter chitin structure on these fungal cells (conditions that will destroy protease activities) still functioned to alter TJ barrier function. Thus, I do not believe that the Belanger reference describes anything that might anticipate the claims of the current application.

12. Specifically, the Belanger reference does not anticipate claim 1 of the present invention because it does not disclose a composition comprising a pharmaceutically active compound and a non-encapsulating adjuvant, wherein the adjuvant comprises a fungal cell or a fragment thereof. Reactivity of a fungal cell or fragment thereof, with chemicals such as BCA and PMSF does not constitute a pharmaceutical application; such an outcome merely demonstrates that chemical modification of a fungal cell or fragments thereof is possible. Pharmaceutical applications require materials with therapeutic qualities and these materials do not fall in that category.

13. Specifically, the Breton reference does not anticipate claim 1 of the present invention because it does not disclose a method that facilitates transport across a mucosal surface of a pharmaceutically active compound that is typically excluded from entering the body at that site. The Breton publication describes the positive effect achieved by the simultaneous administration of a lactic acid bacterium with a carotenoid compound. In one location of the publication, it is stated that yeast might be used to replace the lactic acid bacterium for this application. Although the statement is repeatedly made that this combination provides a "protective and preventative effect of the skin", there is no data presented to suggest (nor is it inferred anywhere that I can find in the publication) that the presence of the lactic acid bacterium somehow improves the uptake of the co-administered carotenoid. Indeed, the carotenoids listed in this publication are frequently found in the diet and are readily absorbed without co-administration of lactic acid bacterium (or yeast). Thus, as one skilled in the art, I find no relevant connection between the Breton publication and an anticipated outcome for fugal cells or their derivatives to open TJ structures to facilitate the uptake of poorly absorbed materials.

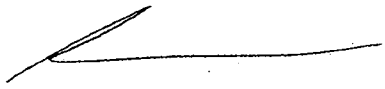
14. The complex combinations of materials described in the Modi publication constitute mixed micelle structures that are claimed to facilitate the transport of large-molecule pharmaceuticals through the oral and nasal membranes. Although some of the materials listed would also be components of fungal cells (e.g. lysine), the vast majority of materials listed in the Modi publication (e.g. cucumber extract) have nothing to do with fungal cells. Further, the invention described in this publication states only oral and nasal membrane application. There is a very important reason for this. The mixed micelle complexes described in the Modi publication are not sufficiently stable to reach the intestinal mucosa in an intact and bioactive form; they can only be sprayed into the nose or into the oral cavity and thus must function only at those sites. The Micap technology describing the use of fungal cells solves this important stability problem by identifying a material and a method to deliver a system to the intestine where it can function to enhance uptake of poorly absorbed materials.

15. The combination of the Breton and Modi references does not obviate claim 1 of the present invention because even in combination, the references fail to disclose OR suggest a composition comprising a pharmaceutically active compound and a non-encapsulating adjuvant, wherein the adjuvant comprises a fungal cell or a fragment thereof, that facilitates uptake of a poorly absorbed material at a mucosal surface. Protection of labile materials by encapsulating them in a probotic structure (e.g. lactic acid bacterium) and identifying methods of opening paracellular junctions are two separate issues. The fact that the present invention finds one material (a fungal cell or fragment adjuvant) capable of achieving both outcomes is truly unique and unexpected as other probiotics (e.g. other probiotics) have not previously been described to achieve this combined outcome.

16. The Molano article describes data obtained using several methods to determine the presence and localization of chitin in the yeast cell wall. This information has no direct bearing on the claims made in the patent application by Micap; the response of a mucosal surface such as the intestinal epithelium to an organism or materials derived from that organism will be due to the sum of potentially additive and neutralizing direct and indirect events that occur as a consequence of this interaction. The work by Molano merely shows that yeast cells express chitin. It does not define or describe the actions of fungal cells or components associated with these cells, such as chitin, would have on mucosal TJ function. As the literature describes, the mere presence of chitin does not automatically result in a modulation of neither TJ function nor its acceptable application for pharmaceutical practices.

17. A review of the literature fails to identify publications suggesting that chitin can alter TJ structure/function. What is found is a series of studies, starting with work from Per Artursson's group (Schipper NG Pharm Res. 1997 Jul;14(7):923-9) that describes the actions of modified forms of chitin, but not chitin itself, having an effect on the TJs of polarized epithelial cells in vitro. Indeed, an entire company has been established that focuses on the application of chemically-derived chitin derivatives (but not the native chitin in a fungal cell!) for the delivery of macromolecules across mucosal surfaces: Kytogenetics, Inc. Additionally, these chemically-modified chitin derivatives can show extreme toxicity when applied to epithelia (Opanasopit P Pharm Dev Technol. 2007;12(5):447-55). It is this combination of facts that makes the application by Micap non-obvious. 1) Only chemically-modified forms of chitin had been shown to affect TJ structures. 2) Fungal cells, as shown by Molano, had chitin at their surface, not these modified forms of chitin. 3) The actions of modified forms of chitin are associated with some level of cytotoxicity presumably due to their actions on epithelial cells to incite disruption of TJ structures. Thus, it is surprising that fungal cells and their derivatives can affect TJ structure and function in a non-toxic manner as shown by the Micap data.

18. I hereby declare that all statements made herein are based on my own knowledge and are true.



Signature

July 29, 2008
Date